INFECTION AND IMMUNITY, Feb. 1996, p. 399–405 Vol. 64, No. 2
0019-9567/96/$04.00+0

Selective Induction of Transforming Growth Factor β in Human Monocytes by Lipoarabinomannan of *Mycobacterium tuberculosis*

KAREN E. DAHL,[1] HIROE SHIRATSUCHI,[1] BEVERLY D. HAMILTON,[1,2] JERROLD J. ELLNER,[1,3] AND ZAHRA TOOSSI[1,2*]

Department of Medicine, Case Western Reserve University,[1] University Hospitals of Cleveland,[3] and Cleveland Veterans Administration Medical Center,[2] Cleveland, Ohio 44106

Received 23 February 1995/Returned for modification 11 May 1995/Accepted 6 November 1995

The induction of macrophage-deactivating (interleukin-10 [IL-10] and transforming growth factor beta [TGF-β]) and macrophage-activating (IL-1, IL-6, and tumor necrosis factor alpha [TNF-α]) cytokines by lipoarabinomannan (LAM) from pathogenic *Mycobacterium tuberculosis* Erdman and H37Rv strains (ManLAM) and nonpathogenic mycobacteria (AraLAM) in human blood monocytes was examined. ManLAM was significantly less potent in induction of TNF-α, IL-1, IL-6, and IL-10 protein and mRNA, whereas its ability to induce TGF-β was similar to that of AraLAM. Differences in induction of TNF-α mRNA by the two LAM preparations only became apparent at late time points of culture (24 h). The induction of TNF-α and IL-1 by purified protein derivative of *M. tuberculosis* was significantly stronger than that by ManLAM. Pretreatment of monocytes with ManLAM did not, however, interfere with cytokine induction by lipopolysaccharide or AraLAM. The extensive mannosyl capping of arabinose termini of ManLAM may underlie the lack of ability to induce some cytokines (IL-1, TNF-α, and IL-10) and the retained ability to induce TGF-β. The latter may have a role in shifting the cytokine milieu in favor of survival of *M. tuberculosis*.

Tuberculosis remains an unresolved public health problem in developing and industrialized countries, accounting for three million deaths annually worldwide (29). Understanding its pathogenesis is therefore of paramount importance, heightened particularly by the recent increase in cases associated with the pandemic of human immunodeficiency virus infection (16). In view of the essential roles played by mononuclear phagocytes in mycobacterial disease and the effects that cytokines have in granuloma formation and immunoregulatory and effector functions, it is important to understand the influence of unique mycobacterial products on cytokine expression.

In this regard, it has been shown, for example, that both *Mycobacterium tuberculosis*-derived purified protein derivative (PPD) and antigen 5, a partially purified cytoplasmic antigen, induce interleukin-1 (IL-1) from human monocytes (34). Similarly, whole organisms of *Mycobacterium bovis*, culture filtrates of *M. tuberculosis* H37Ra, PPD, and antigen 5 all induced production of tumor necrosis factor alpha (TNF-α) from human monocytes and alveolar macrophages (33). Furthermore, a 30-kDa fibronectin-binding protein of *M. tuberculosis* (3) and a 58-kDa antigen in virulent *M. tuberculosis* culture filtrate (35) have been shown to induce TNF-α. We have recently shown that *M. tuberculosis* (17) and its PPD (32) also induce the deactivating cytokine transforming growth factor β (TGF-β).

The expression of cytokines such as TNF-α and IL-1 in response to mycobacterial products is of interest because they are likely to mediate, at least in part, the pathogenesis and clinical manifestations of mycobacterial diseases. In a murine model, TNF-α was shown to have an essential role in the formation and maintenance of granuloma and in the elimination of mycobacteria following *M. bovis* BCG infection (21). Other investigators have demonstrated that TNF-α, when added to infected human monocyte-derived macrophages, augmented intracellular killing of *Mycobacterium avium* in a dose-dependent manner (7). Both TNF-α and gamma interferon decreased and TGF-β increased the intracellular multiplication of *M. tuberculosis* in human monocytes (17). Several groups have shown increased TNF-α production in skin biopsies and in peripheral blood mononuclear cells (PBMC) of patients with the immunologically reactive localized (tuberculoid) form of leprosy (1, 5). TNF-α has also been localized to pleural mononuclear cells of patients with the generally self-contained pleural infection associated with *M. tuberculosis* (6). On the other hand, TGF-β is produced by monocytes of patients with active tuberculosis and is present in tuberculous granulomatous lung lesions of such patients (30).

With this in mind, attention has turned recently to a highly immunogenic surface lipoglycan of mycobacteria known as lipoarabinomannan (LAM). This lipoglycan accounts for up to 5 mg/g of bacterial weight and has been highly purified from *Mycobacterium leprae* and *M. tuberculosis* (19). LAM consists of a mannan core with oligoarabinosyl-containing side chains, which is attached to a phosphatidylinositol anchor at one end (11, 18). LAM has been studied most extensively in murine cells; LAM interferes with gamma interferon-mediated activation of macrophages (10, 28), scavenges toxic oxygen free radicals, inhibits protein kinase C activity (10), and induces the expression of macrophage immediate-early genes (26). LAM has also been shown to induce several cytokines, especially TNF-α, in both murine and human mononuclear phagocytes (4, 13). Some studies have concluded that LAM is the main TNF-inducing component present in whole mycobacteria and preparations thereof, such as PPD.

Two major classes of LAM have been isolated from mycobacteria to date (25); LAM (ManLAM) from the virulent Erdman strain (12), both the virulent H37Rv and the avirulent H37Ra strains, several other pathogenic mycobacteria, and BCG (25) is characterized by extensive mannose capping of the

* Corresponding author. Mailing address: Case Western Reserve University, School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106-4984. Phone: (216) 368-4844. Fax: (216) 368-2034.

arabinan termini. In contrast, the arabinan termini of LAM from a rapidly growing laboratory strain of mycobacteria (AraLAM) is not mannosylated (12). Interestingly, AraLAM was shown to induce up to 100-fold more TNF-α in murine macrophages than ManLAM. This marked biologic difference in activity has been attributed to the heavy mannose capping of ManLAM. Other studies have shown that the phosphatidylinositol anchor, similar between the two LAMs, is partly (13) or wholly (4) responsible for the biologic activity of LAM.

Therefore, in view of the central role which TNF-α appears to play in the immune response to *M. tuberculosis* and data suggesting that ManLAM induced less TNF-α in mouse macrophages than AraLAM, we believed that it was important to see if the latter observations could be extended to human monocytes and whether differential induction of cytokines by the two LAMs included the deactivating cytokines TGF-β and IL-10. We found that LAM from virulent *M. tuberculosis* was a poor inducer of the proinflammatory cytokines TNF-α, IL-1, and IL-6. In contrast, AraLAM and PPD were potent inducers of these cytokines. Both LAMs were poor inducers of the anti-inflammatory cytokine IL-10 but were similar in induction of TGF-β. Also, LAM from *M. tuberculosis* failed to modulate the activation of human monocytes by other stimuli.

MATERIALS AND METHODS

Isolation of primary human monocytes. Heparinized whole blood (20 U/ml) was obtained by venipuncture from healthy, tuberculin skin test-nonreactive donors. PBMC were separated by sedimentation through Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) (8). Monocytes were prepared by suspending PBMC (5×10^6/ml) in RPMI 1640 medium (BioWhittaker, Walkersville, Md.) supplemented with 2 mM L-glutamine and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer and incubated for 1 h at 37°C on plastic petri dishes (100 by 20 mm; Falcon, Oxnard, Calif.) which had been coated with 1.5 ml of pooled human serum (PHS). After removal of the nonadherent cells, the adherent monolayers were dislodged gently with a Teflon scraper, and the cells were counted. The adherent cell population consisted of 85 to 90% monocytes by peroxidase staining (20) and was 99% viable by trypan blue staining.

For RNA studies, monocytes were resuspended (2×10^6/ml) in RPMI with 2% (vol/vol) PHS in petri dishes (100 by 20 mm; Falcon) and incubated at 37°C with 5% CO_2. At specified points after the addition of stimuli, total cellular RNA was obtained as described below. To generate cytokines, monocytes (10^6/ml) in medium containing 2% (vol/vol) PHS were added to petri dishes (35 by 10 mm; Falcon), and cell supernatants were collected and kept at −20°C. In some experiments, cell lysates were prepared by addition of 0.5 ml of sterile water to plates, followed by three cycles of freeze-thawing and final reconstitution with 0.5 ml of phosphate-buffered saline (PBS) and 4% (vol/vol) PHS before storage.

Reagents. Lipopolysaccharide (LPS) derived from *Escherichia coli* F583 (Sigma) was dissolved in RPMI and kept frozen at −20°C. Prior to use, the stock was thawed and vortexed vigorously for 5 min. PPD of *M. tuberculosis* was a gift from Lederle Laboratories (American Cyanamid, Inc., Wayne, N.J.). ManLAM from the virulent strains of *M. tuberculosis* Erdman and H37Rv and AraLAM from a rapidly growing laboratory strain of mycobacterium were provided by Patrick Brennan (Colorado State University, Ft. Collins). Details of the extraction procedure and structures of the molecules have been published elsewhere (12, 18, 19). Each preparation was passed over a Detoxigel column to deplete endotoxin contamination (19). The endotoxin content of all reagents was measured in a chromogenic *Limulus* lysate assay (BioWhittaker). The two LAM preparations contained insignificant amounts of endotoxin (<20 pg/mg). PPD had 16 ng of endotoxin per mg of protein. The LAM content of PPD was assessed by Western immunoblot analysis with monoclonal antibody to LAM (CS-35), which recognizes LAMs from all strains of *M. tuberculosis* (25), and was found to be less than 1 μg/100 μg of PPD.

Isolation and analysis of total cellular RNA. Total cellular RNA was isolated by the guanidinium cesium method (31). RNA (5 to 10 μg) was electrophoresed through 0.8% agarose–2 M formaldehyde gels and transferred to nylon membranes (Magnagraph; Micron Separations, Inc.). Membranes were baked for 1 h at 80°C and prehybridized for at least 5 h in 1% fatty acid-free bovine serum albumin (BSA; Sigma)–0.2 M sodium phosphate (pH 7.2)–3.75 M formamide–0.001 M EDTA–7% sodium dodecyl sulfate (SDS). [^{32}P]dCTP cDNA probes were prepared by random priming and added directly to the prehybridization solution at 10^6 cpm/ml, and the membranes were incubated overnight in a shaking water bath at 65°C. Membranes were washed once in 2× SSC–2% SDS for 30 min at 50°C and twice in 0.2× SSC–0.2% SDS for 30 min at 50 and 65°C successively (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate).

Probes were generated from plasmid preparations made per the instructions provided with a kit (Qiagen, Chatsworth, Calif.) and included the following: (i) a 370-bp *Bam*HI-*Hin*dIII fragment from the human TNF-α clone pAW783; (ii) a 550-bp *Bam*HI-*Nde*I fragment from the human IL-1β plasmid pΔ11; (iii) a 1,200-bp *Eco*RI fragment from the human IL-6 plasmid pXM309; (iv) a 760-bp *Bgl*II-*Hin*dIII fragment from the human IL-10 plasmid pCDSRα, provided by Kevin Moore (DNAX Research Institute, Palo Alto, Calif.); and (v) a 2,000-bp *Eco*RI fragment from the human TGF-β plasmid (J. Bell, University of Illinois). To assess equal loading of RNA, membranes were stripped and reprobed with a riboprobe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (American Type Culture Collection).

Cytokine assays. Enzyme-linked immunosorbent assays (ELISAs) were performed in 96-well Immulon 4 plates (Fisher). For the TNF-α ELISA, the murine monoclonal anti-human TNF-α antibody clone F12 (Olympus Immunochemicals, Lake Success, N.Y.) was used as the coating antibody and a polyclonal rabbit anti-human TNF-α antibody (Genzyme) was used as the capping antibody. The TGF-β ELISA was performed with the murine monoclonal anti-human TGF-β antibody 12H5 (gift of Genentech) (22) as the coating antibody and a polyclonal chicken anti-human TGF-β antibody (R&D Systems, Minneapolis, Minn.) as the capping antibody. Samples were treated with 1 N hydrochloric acid for 30 min and neutralized with NaOH-HEPES before assay. The IL-10 ELISA was performed with a pair of monoclonal anti-human IL-10 antibodies, available from Pharmingen (San Diego, Calif.). Human IL-1β and IL-6 were assayed with commercial ELISA kits (R&D Systems).

RESULTS

Induction of TNF-α, IL-1β, and IL-6 by mycobacterial LAM preparations. In preliminary studies, the induction of TNF-α in human monocytes by the different preparations of LAM was examined. Monocytes from three donors were stimulated for 24 h with a range of concentrations (0.1, 1, and 10 μg/ml) of each LAM. Whereas AraLAM induced TNF-α at all concentrations tested, induction was maximal at 10 μg/ml. Insignificant TNF-α was induced by ManLAM at all concentrations tested; however, induction was best at 10 μg/ml (data not shown). Monocytes from a total of nine donors were stimulated with AraLAM, ManLAM, and LPS, each at 10 μg/ml, and culture supernatants were harvested at 24 h. AraLAM induced 3.4-fold-greater concentrations of immunoreactive TNF-α than did ManLAM ($P < 0.01$) (Fig. 1A). However, AraLAM induced less TNF-α than LPS ($P < 0.001$). To ensure that altered kinetics of induction of TNF-α did not account for the differences seen, monocytes were stimulated for 1, 2, 4, 6, 16, 24, and 48 h with ManLAM or AraLAM. Supernatant TNF-α activity remained lower in ManLAM-induced monocyte cultures at all time points (data not shown). In each experiment, TNF-α activity in cell lysates was assessed in parallel with cell supernatants to ensure that differences in induction of TNF-α were not secondary to differences in compartmentalization of the cytokine. For both LAM preparations, the amount of TNF-α in cell lysates at all time points was insignificant and about 1 to 2% of supernatant TNF-α activity (data not shown).

Next, the production of IL-1β and IL-6 in response to both LAM preparations was analyzed in monocytes cultured from five donors. AraLAM induced from 1.9- to 17.1-fold more IL-1 than did ManLAM (mean fold difference, 8.1) ($P < 0.01$). Similarly, AraLAM induced from 1.2- to 4.1-fold more IL-6 (mean fold difference, 2.6) than did ManLAM.

To examine the basis for the differences in cytokine production by the two preparations of LAM, we next assessed the induction of cytokine-specific mRNA in monocyte cultures stimulated with AraLAM, ManLAM, and LPS. The induction of TNF-α mRNA by the two LAM preparations was comparable at 3 h and lower than that by LPS (Fig. 1B). However, steady-state TNF-α mRNA levels induced by ManLAM were lower than those with AraLAM and LPS at 24 h of culture. By contrast, the induction of IL-1β and IL-6 by ManLAM was minimal at both early (3 h) and late (24 h) time points (Fig. 1C).

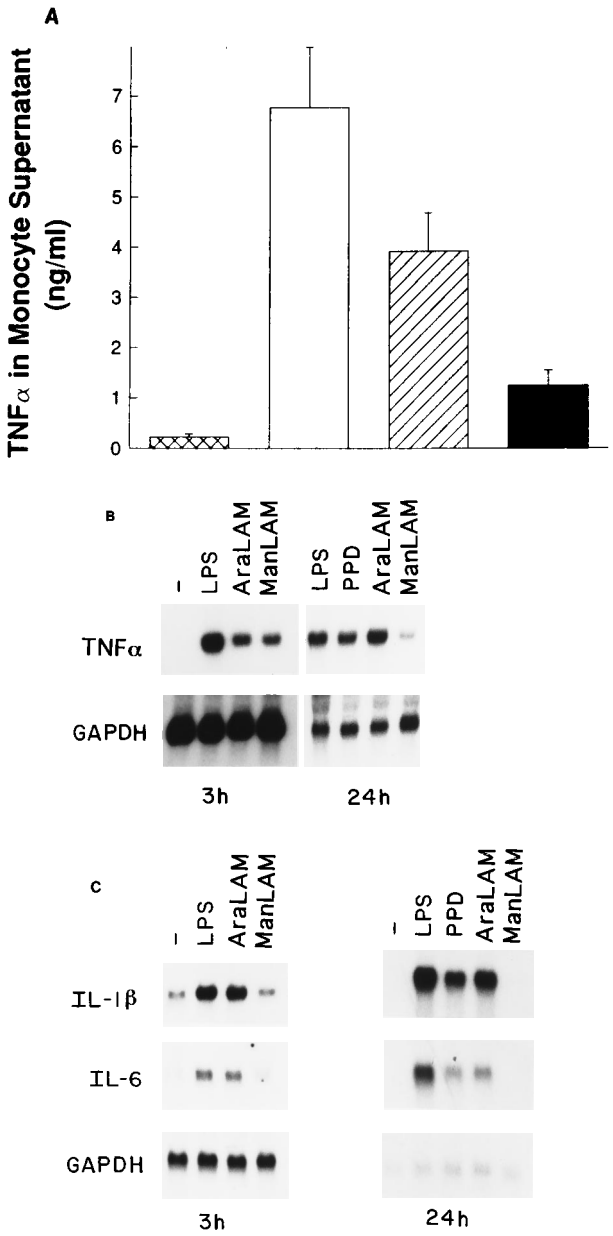

FIG. 1. Expression of proinflammatory cytokines in monocyte cultures. Monocytes were cultured with LPS, AraLAM, ManLAM, or PPD of *M. tuberculosis* at 10 μg/ml each. Cytokine activity was measured in culture supernatants, and cytokine-specific mRNA was assessed in cell lysates. (A) TNF-α concentration in 24-h culture supernatants by ELISA. Cross-hatched bar, medium; open bar, LPS; hatched bar, AraLAM; solid bar, ManLAM. Data represent the mean ± standard error of the mean (SEM) of nine experiments. For AraLAM versus ManLAM, $P < 0.01$. (B) Northern (RNA blot) analysis of TNF-α expression in monocytes at 3 h (left) and 24 h (right) of culture. (C) Northern blot analysis of IL-1β and IL-6 expression at 3 h (left) and 24 h (right) of culture. In panels B and C, stripped blots were reprobed for GAPDH expression.

Induction of cytokines by PPD and LAM of *M. tuberculosis*. PPD is prepared from a crude autoclaved preparation of the culture filtrate of virulent *M. tuberculosis* and contains both peptide and nonpeptide mycobacterial antigens. The cytokine-inducing capacity of PPD has, however, been attributed in part to its LAM content. We therefore compared the capacity of ManLAM and PPD to induce IL-1 and TNF-α. The PPD used in these studies had less than 1 μg of ManLAM per 100 μg of protein, as assessed by Western blot analysis with anti-LAM antibody (Materials and Methods). Monocytes were cultured with ManLAM or PPD at 10 μg/ml or in medium alone. Supernatants were collected after 24 h and assessed for TNF-α and IL-1 activity. In four experiments, PPD induced significantly more TNF-α (4.7-fold, $P < 0.05$) and IL-1 (8.8-fold, $P < 0.05$) than ManLAM. In parallel, induction of TNF-α, IL-1, and IL-6 mRNA by PPD was higher than by ManLAM (Fig. 1B and C).

Induction of IL-10 and TGF-β by mycobacterial LAM. We next examined the induction of the anti-inflammatory cytokines IL-10 and TGF-β by AraLAM and ManLAM in monocytes. Monocytes were cultured with each preparation of LAM or LPS at 10 μg/ml. IL-10 and TGF-β were assessed by ELISA in 24-h culture supernatants. Total cellular RNA was assessed for cytokine expression. Both LAM preparations induced IL-10 poorly. Again, AraLAM induced about fivefold-higher amounts of IL-10 than ManLAM (mean, 350 ± 117 pg/ml versus 65 ± 43 pg/ml) ($P < 0.02$). The ability of AraLAM to induce IL-10 in monocyte cultures was sixfold lower than that of LPS ($P < 0.02$) (Fig. 2A). In parallel, the induction of IL-10 mRNA by the two LAM preparations was lower than that by LPS. However, ManLAM was less potent in induction of IL-10 mRNA than AraLAM (Fig. 2B). On the other hand, the two LAM preparations induced comparable amounts of TGF-β protein in culture, which was about 50% of LPS-induced cytokine activity (Fig. 2C). ManLAM induced TGF-β in monocytes by twofold in seven separate experiments ($P < 0.05$). Furthermore, in three other experiments, ManLAM extracted from another virulent strain of *M. tuberculosis*, H37Rv, induced amounts of TGF-β similar to those induced by Erdman ManLAM (1.1 ± 0.3 ng/ml and 1.4 ± 0.4 ng/ml, respectively). The constitutive expression of TGF-β mRNA was not affected by either ManLAM or AraLAM (Fig. 2D).

Effect of ManLAM on induction of cytokines. Most of the biological activity of LAM has been attributed to the phosphatidylinositol end of the molecule, which is identical in AraLAM and ManLAM (4, 13). Considering the differences in monocyte activation, it was possible, however, that a different structural part of ManLAM was suppressive to the induction of protein synthesis induced by the activating moiety. Recently, mannose receptors have been shown to be important in phagocytosis of the virulent Erdman strain (27). We speculated that engagement of the mannosyl residues of ManLAM with their respective receptors could potentially lead to a reduction in monocyte activation. To examine this issue, monocytes were cultured in the presence of ManLAM (10 μg/ml), AraLAM (10 μg/ml), or medium for 1 h at 37°C, after which suboptimal concentrations of LPS (0.5 μg/ml) were added to cultures. No reduction in subsequent expression of cytokine mRNA (data not shown) or supernatant TNF or IL-1 activity upon stimulation with LPS (Fig. 3) was apparent. Furthermore, both ManLAM and AraLAM enhanced LPS-induced cytokine production marginally. Similarly, ManLAM pretreatment did not reduce induction of cytokines by AraLAM (data not shown).

DISCUSSION

This study indicates that ManLAM from virulent *M. tuberculosis* has a lower capacity to activate human monocytes to produce proinflammatory (TNF-α, IL-1, and IL-6) and some anti-inflammatory (IL-10) cytokines, in contrast to preserved induction of TGF-β, than the structurally similar but not identical AraLAM from a rapidly growing mycobacterial strain. The differences in cytokine induction by AraLAM and ManLAM may be attributed to variations in the nonreducing ter-

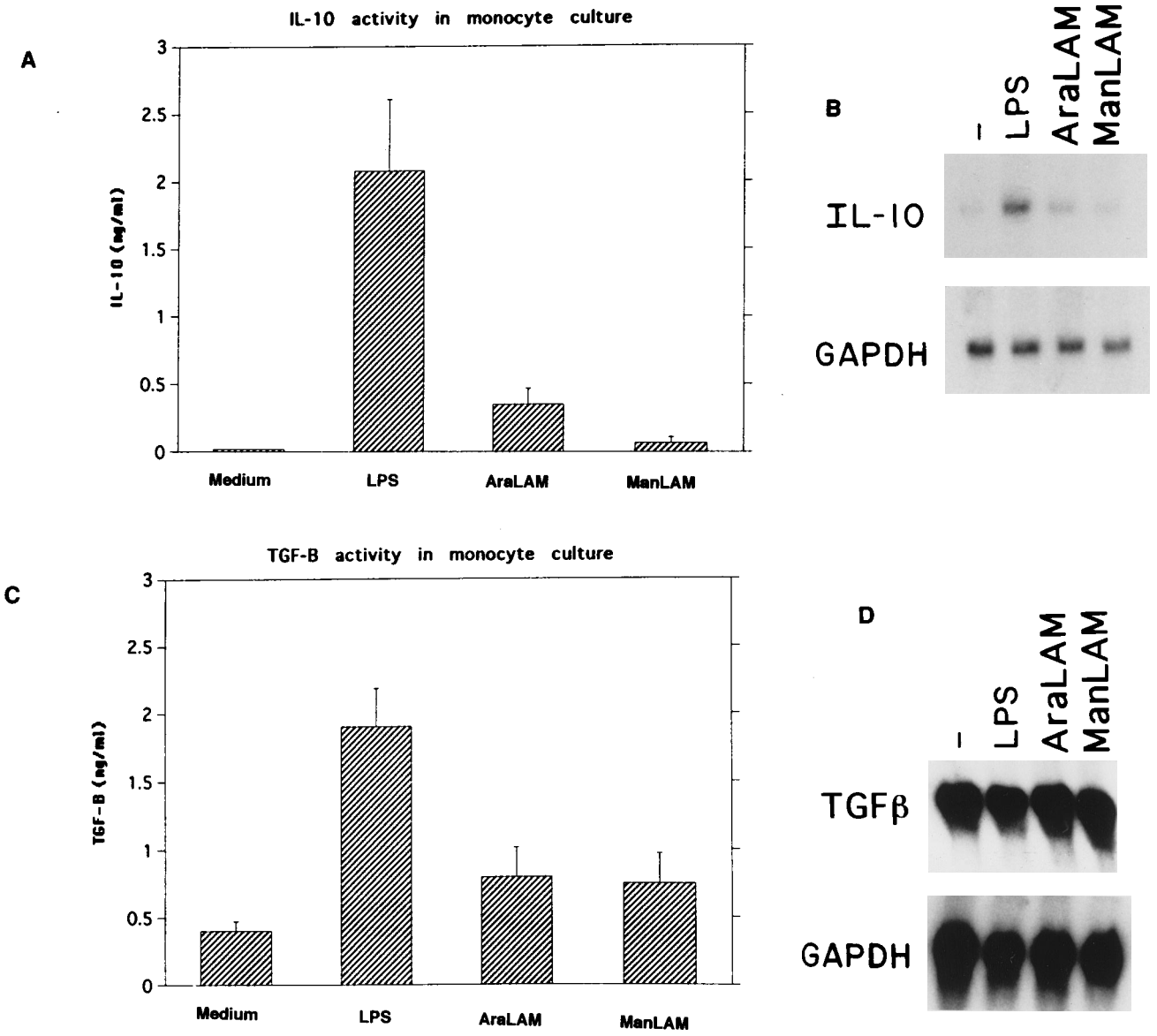

FIG. 2. Induction of IL-10 and TGF-β in monocytes. Monocytes were cultured with LPS, AraLAM, or ManLAM at 10 μg/ml or with medium. Culture supernatants were assessed for IL-10 (A) and TGF-β (C) by ELISA. Total cellular RNA was assessed for IL-10 (B) and TGF-β (D) mRNA by Northern blot analysis. The data in panels A and C represent the mean ± SEM of six experiments.

mini of the two molecules. Furthermore, the ManLAM of *M. tuberculosis*, although present in small amounts in PPD (0.1 μg/mg), is not the sole or the most active cytokine-inducing moiety of PPD, nor is it suppressive to cytokine induction by bacterial LPS or other mycobacterial products.

Recently, LAM of the mycobacterial cell wall has been ascribed a major role in host-bacterium interactions and considered a key pathogenic factor (10, 26, 28). Murine peritoneal macrophages infected with *M. leprae* or treated with LAM were not responsive to activation by gamma interferon in production of reactive oxygen intermediaries and expression of class II major histocompatibility complex molecules (28). In parallel, LAM scavenged oxygen radicals, inhibited protein kinase C activity, and restricted gamma interferon-mediated transcriptional activation of human monocytic cell lines (10). More recently, direct comparisons between ManLAM from the pathogenic Erdman strain and AraLAM have shown a lower induction by the former of the immediate-early genes, such as c-*fos*, KC, and JE (26), and TNF-α secretion in murine macrophages (13). Also, in human monocytic cell-line THP-1, AraLAM induced higher concentrations of immunoreactive TNF-α and IL-1β, while cytokine induction by ManLAM was insignificant (36). Furthermore, it was shown that AraLAM activated THP-1 cells in a manner analogous to LPS, using the LPS receptor CD14 to induce cytokines in whole blood cells (36). Our studies extend these observations to human monocyte expression of macrophage-activating and -deactivating molecules by LAM. ManLAM induced significantly less TNF-α (3.4-fold), IL-1β (8.1-fold), and IL-6 (2.6-fold) than AraLAM. In parallel, the induction of IL-1β and IL-6 mRNA was significantly lower in ManLAM-stimulated monocyte cultures at early (3 h) and late (24 h) time points. The induction of TNF-α mRNA by ManLAM was similar to that by AraLAM at 3 h but significantly less after longer culture (24 h). These

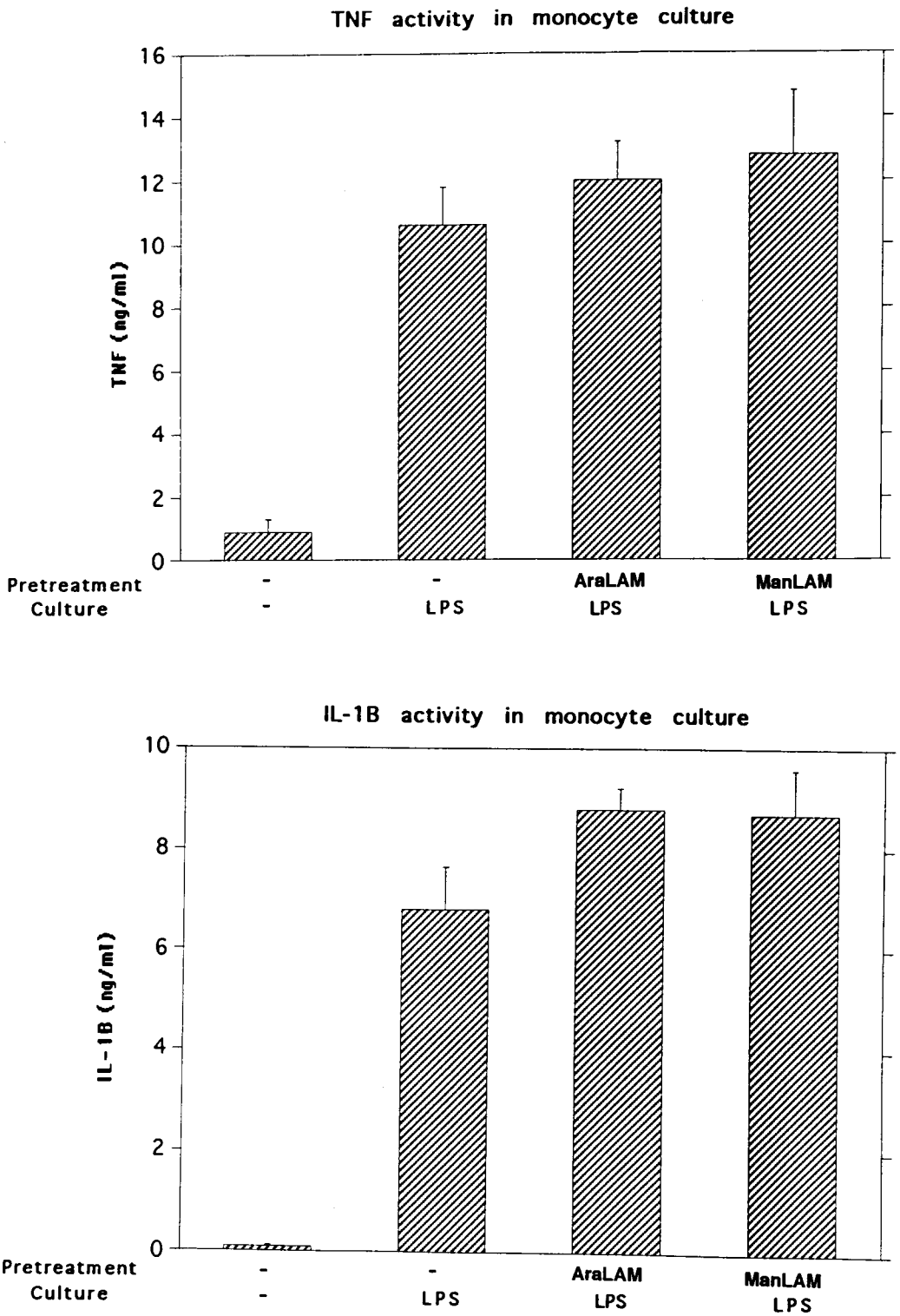

FIG. 3. Effect of LAM on LPS-stimulated cytokine production in monocytes. Cells were treated with ManLAM (10 μg/ml), AraLAM (10 μg/ml), or medium for 1 h, and then cultures received LPS at 0.5 U/ml or medium. Culture supernatants were assessed for TNF-α (top) and IL-1β (bottom) activity at 24 h. Data represent the mean ± SEM of five experiments.

differences in the pattern of cytokine responses to ManLAM at early time points of monocyte induction may be due to the sensitivity of expression of the TNF-α gene to short-lived repressor proteins (14), the production of which may be inhibited by ManLAM. Thus, it appears that unlike murine macrophages (26), in human monocytes, transcriptional activation of early genes such as that for TNF-α is not downregulated by ManLAM. Since both transcriptional and posttranscriptional

activation is operative in induction of TNF-α (9), the effect of ManLAM on monocytes may be due to inefficient translation of TNF-α at early time points. At later time points, however, a defect in transcription of TNF-α is superimposed.

By contrast, the differences between ManLAM and Ara LAM with regard to induction of the deactivating cytokines IL-10 and TGF-β were less marked. Both preparations induced IL-10 mRNA and protein poorly compared with LPS. However, once again, ManLAM induced less protein and mRNA than AraLAM. On the other hand, induction of TGF-β by the two LAMs was similar and approximately half of the induction by LPS. Furthermore, ManLAM from a second virulent laboratory strain, H37Rv, was comparable to ManLAM from Erdman strain in induction of TGF-β. On the other hand, TGF-β mRNA was not modulated by ManLAM or AraLAM. Therefore, as with LPS-induced TGF-β production (2), induction of TGF-β protein by LAM is not due to transcriptional activation. By contrast, PPD of *M. tuberculosis* induced TGF-β transcription (32).

We found that ManLAM did not suppress the induction of cytokines (IL-1β and TNF-α) in monocytes by LPS or Ara LAM. Furthermore, LPS-induced TNF-α and IL-1β levels increased marginally and to the same extent when monocytes were pretreated with either ManLAM or AraLAM (Fig. 3). Since AraLAM may induce monocytes through the LPS-binding molecule CD14, these data may indicate a maximal usage of these receptors by LPS and AraLAM in culture. Alternatively, displacement of AraLAM bound to CD14 by LPS in the second culture could account for these results. However, unlike the suppression of gamma interferon-induced macrophage activation by LAM reported previously (10, 24, 28), we did not find a direct downregulation of monocyte activation, in terms of production of cytokines, by ManLAM. Differences in the amounts of LAM used could underlie these discordant findings. Both LAMs were used at lower amounts (10 μg/ml) in this study compared with the 10 to 10,000 μg/ml in that by Sibley et al. (28) and Moreno et al. (24). Furthermore, Molloy et al. found that suppression of T-cell blastogenesis attributed to LAM from *M. leprae* and *M. tuberculosis* was due to the presence of LPS in their preparations (23). The role of ManLAM in the virulence and pathogenicity of *M. tuberculosis* is presently uncertain. Clearly, the cell walls of both fully virulent (Erdman and H37Rv) and attenuated (BCG and H37Ra) mycobacteria contain ManLAM (25). Although mannose capping cannot be the sole determinant of pathogenicity, it may have some role through interactions with other, presumably multiple, virulence determinants. In this regard, the finding that ManLAM retains the capacity to induce TGF-β, a key immunosuppressive and macrophage-deactivating molecule, is certainly consonant with a role in immunopathogenesis. TGF-β induced by ManLAM might, in turn, block T-cell-dependent monocyte activation, thus favoring intracellular bacillary replication.

Since LAM is ubiquitous in mycobacterial preparations, it was important to show that some of the reported monocyte-activating attributes of these preparations, in particular PPD, were not just due to contamination with LAM. The ManLAM content of PPD was less than 1 μg/100 μg of protein by Western blot analysis. In direct comparison with PPD of virulent *M. tuberculosis*, ManLAM proved to induce significantly less TNF-α, IL-1β, and IL-6 protein and cytokine-specific mRNA. Induction of TNF-α by purified antigens of *M. tuberculosis*, such as the 58-kDa (34) and 30-kDa alpha (3) antigens, is therefore unlikely to be due just to contamination with LAM.

In conclusion, although LAM from *M. tuberculosis* activates human monocyte cytokines poorly, it is not suppressive of monocyte production of cytokines. On the other hand, the retained ability of ManLAM to induce TGF-β may shift the cytokine milieu induced by *M. tuberculosis* and its components to one of macrophage deactivation in situ and thereby promote survival of the organism.

ACKNOWLEDGMENTS

This study was supported by a medical research grant from the Department of Veterans Affairs and by National Institute of Health grant AI 18471.

We thank D. Chatterjee and J. T. Belisle for supplying purified LAM preparations (NIH/NIAID contract AI 25147 to Colorado State University) and P. Brennan for useful comments on the manuscript.

REFERENCES

1. **Arnoldi, J., J. Gerdes, and H. D. Flad.** 1990. Immunohistologic assessment of cytokine production of infiltrating cells in various forms of leprosy. Am. J. Pathol. **137:**749–753.
2. **Assoian, R. K., B. E. Fleurbelys, H. C. Stevenson, P. J. Miller, D. K. Madtes, E. W. Raines, R. Ross, and M. B. Sporn.** 1987. Expression and secretion of type β transforming growth factor by activated human macrophages. Proc. Natl. Acad. Sci. USA **84:**6020–6029.
3. **Averill, L. E., Z. Toossi, H. Aung, and J. J. Ellner.** 1993. Regulation of tumor necrosis factor (TNF) production in human monocytes in response to the 30 kD antigen and purified protein derivative (PPD) of *Mycobacterium tuberculosis*. TB Scientific Panel, U.S.-Japan Cooperative Medical Sciences Program. National Institutes of Health, Bethesda, Md.
4. **Barnes, P. F., D. Chatterjee, J. S. Abrams, S. Lu, E. Wang, M. Yamamura, P. J. Brennan, and R. L. Modlin.** 1992. Cytokine production induced by *Mycobacterium tuberculosis* lipoarabinomannan. J. Immunol. **149:**541–547.
5. **Barnes, P. F., D. Chatterjee, P. J. Brennan, T. H. Rea, and R. L. Modlin.** 1992. Tumor necrosis factor production in patients with leprosy. Infect. Immun. **60:**1441–1446.
6. **Barnes, P. F., S.-J. Fong, P. J. Brennan, P. E. Twomey, A. Mazumder, and R. L. Modlin.** 1990. Local production of tumor necrosis factor and IFNγ in tuberculous pleuritis. J. Immunol. **145:**149–154.
7. **Bermudez, L. E., and L. S. Young.** 1988. Recombinant tumor necrosis factor alone or in combination with interleukin-2 but not gamma-interferon is associated with killing of *M. avium* from AIDS patients. J. Immunol. **140:** 3006–3013.
8. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. **21**(Suppl. 97)**:**9–29.
9. **Burchett, S. K., W. M. Weaver, J. A. Westall, S. Kronheim, and C. B. Wilson.** 1988. Regulation of tumor necrosis factor/cachectin and IL-1 secretion in human mononuclear phagocytes. J. Immunol. **140:**3473–3479.
10. **Chan, J., X. Fan, S. W. Hunter, P. J. Brennan, and B. R. Bloom.** 1991. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. Infect. Immun. **59:**1755–1761.
11. **Chatterjee, D., C. M. Bozic, M. McNeil, and P. J. Brennan.** 1991. Structural features of the arabinan component of the lipoarabinomannan of *Mycobacterium tuberculosis*. J. Biol. Chem. **266:**9652–9660.
12. **Chatterjee, D., K. Lowell, B. Rivoire, M. R. McNeil, and P. J. Brennan.** 1992. Lipoarabinomannan of *Mycobacterium tuberculosis*: capping with mannosyl residues in some strains. J. Biol. Chem. **267:**6234–6239.
13. **Chatterjee, D., A. D. Roberts, K. Lowell, P. J. Brennan, and I. M. Orme.** 1992. Structural basis of capacity of lipoarabinomannan to induce secretion of tumor necrosis factor. Infect. Immun. **60:**1249–1253.
14. **Collart, M. A., D. Belin, J. D. Vasalli, S. Kossodo, and P. Vasalli.** 1986. γ interferon enhanced macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes which are controlled by short-lived repressors. J. Exp. Med. **164:**2113.
15. **Dinarello, C. A.** 1991. The proinflammatory cytokines IL-1 and TNFα and treatment of the septic shock syndrome. J. Infect. Dis. **163:**1177–1182.
16. **Ellner, J. J.** 1990. Tuberculosis in the time of AIDS: the facts and message. Chest **98:**105–108.
17. **Hirsch, C. S., T. Yoneda, L. E. Averill, J. J. Ellner, and Z. Toossi.** 1994. Enhancement of intracellular growth of *Mycobacterium tuberculosis* in human monocytes by transforming growth factor β1. J. Infect. Dis. **170:**1229–1237.
18. **Hunter, S. W., and P. J. Brennan.** 1990. Evidence for the presence of a phosphatidylinositol anchor on lipoarabinomannan and lipomannan of *M. tuberculosis*. J. Biol. Chem. **267:**9272–9279.
19. **Hunter, S. W., H. Gaylord, and P. J. Brennan.** 1986. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. J. Biol. Chem. **261:**12345–12351.
20. **Kaplow, L.** 1965. Simplified myeloperoxidase staining using benzidine hydrochloride. Blood **26:**215–219.
21. **Kindler, V., A. P. Sappino, G. E. Grau, P. F. Piguet, and P. Vassalli.** 1989.

The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell **56:**731–740.
22. **Lucas, C., L. N. Bald, B. M. Fendly, M. Mora-Worms, I. S. Figari, E. J. Patzer, and M. A. Palladino.** 1990. The autocrine production of transforming growth factor-β1 during lymphocyte activation. J. Immunol. **145:**1415–1421.
23. **Molloy, A., G. Gaudernack, W. R. Levis, Z. A. Cohn, and G. Kaplan.** 1990. Suppression of T-cell proliferation by *M. leprae* and its products: the role of lipopolysaccharide. Proc. Natl. Acad. Sci. USA **87:**973–977.
24. **Moreno, C., J. Taverne, A. Mehlert, C. A. W. Bate, R. J. Brealey, A. Meager, G. A. W. Rook, and J. H. L. Playfair.** 1989. Lipoarabinomannan from *Mycobacterium tuberculosis* induces the production of tumor necrosis factor from human and murine macrophages. Clin. Exp. Immunol. **76:**240–245.
25. **Prinzis, S., D. Chatterjee, and P. J. Brennan.** 1993. Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. J. Gen. Microbiol. **139:**2649–2658.
26. **Roach, T. I., H. C. Barton, D. Chatterjee, and J. M. Blackwell.** 1993. Macrophage activation: liporabinomannan from avirulent and virulent strains of *M. tuberculosis* differentially induces the early genes c-fos, KC, JE, and tumor necrosis factor-α. J. Immunol. **150:**1886–1896.
27. **Schlesinger, L. S.** 1993. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. J. Immunol. **150:**2920–2930.
28. **Sibley, L. D., L. B. Adams, and J. L. Krahenbuhl.** 1990. Inhibition of interferon-gamma-mediated activation in mouse macrophages treated with lipoarabinomannan. Clin. Exp. Immunol. **80:**141–148.
29. **Styblo, K.** 1989. Overview and epidemiologic assessment of the current global tuberculosis situation with an emphasis on control in developing countries. Rev. Infect. Dis. **11:**5339–5344.
30. **Toossi, Z., P. Gogate, H. Shiratsuchi, T. Young, and J. J. Ellner.** 1995. Enhanced production of TGFβ by blood monocytes from patients with active tuberculosis and presence of TGFβ in tuberculous granulomatous lung lesions. J. Immunol. **154:**465–473.
31. **Toossi, Z., J. Lapurga, R. Ondash, J. Sedor, and J. J. Ellner.** 1990. Expression of functional IL-2 receptors by peripheral blood monocytes from patients with active pulmonary tuberculosis. J. Clin. Invest. **85:**1777–1784.
32. **Toossi, Z., T.-G. Young, L. E. Averill, B. D. Hamilton, H. Shiratsuchi, and J. J. Ellner.** 1995. Induction of transforming growth factor β1 by purified protein derivative of Mycobacterium tuberculosis. Infect. Immun. **63:**224–228.
33. **Valone, S. E., E. A. Rich, R. S. Wallis, and J. J. Ellner.** 1988. Expression of tumor necrosis factor in vitro by human mononuclear phagocytes stimulated with whole *Mycobacterium bovis* BCG and mycobacterial antigens. Infect. Immun. **56:**3313–3315.
34. **Wallis, R. S., H. Fujiwara, and J. J. Ellner.** 1986. Direct stimulation of monocyte release of interleukin-1 by mycobacterial protein antigens. J. Immunol. **136:**193–196.
35. **Wallis, R. S., R. Paranjape, and M. Philips.** 1993. Identification by two-dimensional gel electrophoresis of a 58-kilodalton tumor necrosis factor-inducing protein of *Mycobacterium tuberculosis*. Infect. Immun. **61:**627–633.
36. **Zhang, Y., M. Doerfler, C. Theodore, B. Guillemin, and W. N. Rom.** 1993. Mechanisms of stimulation of interleukin-1β and tumor necrosis factor-α by mycobacterium tuberculosis components. J. Clin. Invest. **91:**2076–2083.

Editor: R. E. McCallum